ORAL PRESENTATION ABSTRACTS

*Sex Differences in the Endocannabinoid System Determines Differences in Juvenile Rough-and-Tumble Play Behavior and Correlating Differences in Phagoptosis by Microglia in the Developing Amygdala

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The amygdala is a sexually dimorphic brain region that is critical for the expression of social behavior. In rats, the medial amygdala mediates the sex difference in juvenile rat play behavior. During early postnatal development, the male amygdala has a higher endocannabinoid (ECB) tone and contains fewer newborn cells than females, which correlates to increased levels of juvenile rough-and-tumble play. Masculinization of the female developing amygdala through agonism of the CB1 and CB2 ECB receptors, decreases the number of newborn cells and increases subsequent juvenile rough-and-tumble play (Krebs-Kraft et al. 2010 and Argue et al. 2017). We now report that the higher ECB tone in males is due to the actions of testosterone. Treating neonatal females with exogenous testosterone was sufficient to increase the ECB tone and juvenile rough-and-tumble play. This testosterone-induced increase in female rough-and-tumble play could be ablated with neonatal administration of CB1 and CB2 receptor antagonists. Additionally, microglia, the resident immune cells of the brain, are more phagocytic in the amygdala of males during this postnatal window, suggesting a possible mechanism by which ECBs affect the number of newborn cells. We find that males have more phagocytic microglia between postnatal day 0 and 4, during which time they also have higher ECB tone than females. Increasing ECB tone in female pups by treatment with specific agonists for either CB1 or CB2 receptors increases the number of phagocytic microglia to that of males and results in a corresponding decrease in the number of newborn cells indicated by BrdU labeling. We hypothesize that microglia control the number of newborn cells in the postnatal rat amygdala by phagoptosing (targeted phagocytosis of viable cells) newborn cells in an ECB-dependent manner. Masculinizing female pups with testosterone increases the number of phagocytic microglia and decreases the number of BrdU+ cells. Immunohistochemical analysis together with confocal microscopy indicates phagocytic microglia contain markers of DNA as well as newly proliferated cells in their phagocytic cups. To directly implicate microglia phagoptosis we utilized a complement receptor 3 (CR3) function-blocking antibody to inhibit phagocytosis, which increased the number of BrdU+ cells in both males and females. Thus microglia actively control developmental sex differences in cell genesis and this correlates with sex differences in later life social behavior. We are currently investigating whether juvenile rough-and-tumble play behavior can be mediated through direct manipulation of the microglia during the neonatal period.

This oral presentation will also be a poster presentation.

Sex Differences in Vaccine-Induced Immune Responses

Nicole E. Basta, PhD
University of Minnesota

Understanding the distribution, causes, and consequences of heterogeneity in vaccine-induced immune responses is critical to ensuring that protective levels of immunity persist over time. Sex differences in immune responses following pneumococcal, influenza, measles, and other vaccinations have been reported and may contribute significantly to the variation observed. Yet, for many recently developed vaccines, little is known about whether sex differences are an important driver of variation in immune responses. We designed and implemented a longitudinal study, launched in 2012 in Bamako, Mali, to evaluate changes in immune responses following the introduction of the new meningococcal A polysaccharide-tetanus toxoid conjugate vaccine (MenAfriVac). We investigated sex-specific differences in immune responses two years after vaccination and found that females had higher meningococcal A-specific serum bactericidal antibody titers, a marker of functional immunity and the accepted correlate of protection, in all age groups <18 years. In addition, we demonstrated that while MenAfriVac vaccination boosts tetanus toxoid IgG, a potentially important benefit in countries where maternal and neonatal tetanus remains a concern, sex-specific differences in tetanus immunity before and after MenAfriVac introduction suggest the need for additional, targeted vaccination. Our ongoing research is aimed at investigating the relationship between vaccine-induced immunity and nutritional status and markers of inflammation to determine whether these factors contribute to the sex differences observed.

The Influence of Metabolic Syndrome and Sex on the DNA Methylome in Schizophrenia

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INTRODUCTION: The rate of metabolic syndrome within schizophrenia is 2-3 times higher compared to the general population, which may be partly due to the metabolic side effects of atypical antipsychotics (AAPs). The mechanism by which AAPs increase the risk of metabolic syndrome is not completely known; however, previous work suggests that the folate system, which is involved in the production of methyl groups used in DNA methylation, may be involved. Within this study, the DNA methylome was profiled to identify altered methylation associated with metabolic syndrome in a schizophrenia population and based on sex.

METHODS: Cross-sectional peripheral blood from schizophrenia subjects were utilized for DNA methylation analyses. Discovery analyses (n=49 males and n=47 females) were performed using an epigenome-wide analysis on the Illumina HumanMethylation450 BeadChip. The top hits were analyzed in an additional validation set (n=166) using the gold-standard of site-specific methylation pyrosequencing.

RESULTS: Differential methylation was found within the MAP3K13 gene in females and the CCDC8 gene in males. Significant differences in methylation were validated for the MAP3K13 genes, but not CCDC8, in the validation sample set.

CONCLUSIONS: Differential DNA methylation was identified based on metabolic syndrome, which was also specific to sex. Further prospective work is needed to determine whether DNA methylation could serve as a useful biomarker or treatment target for metabolic syndrome associated with antipsychotic use.

Control of Autoimmunity by Genes, Sex and the Microbiome

Jayne Danska, PhD
The Hospital for Sick Children

Immune-mediated diseases, including type 1 diabetes (T1D), multiple sclerosis, and inflammatory bowel disease, result from the interaction between genetic variants at many loci with poorly understood environmental factors. Over the past 60 years, multiple immune-mediated diseases have seen a dramatic rise in frequency. For example, T1D has increased by >500% in developed countries over this period. In addition to environmental risk factors, the incidences of many autoimmune syndromes display sex bias in incidence and severity, but the mechanisms of sexually dimorphic immune regulation are poorly understood. In the majority of rodent models of autoimmunity, only females are studied because they display more severe effects.

The non-obese diabetic (NOD) mouse displays spontaneous, immune-mediated pancreatic beta cell destruction causing diabetes with both a genetic and environmental etiology. Disease in the NOD mouse is twice as frequent in females compared to males and is also impacted by with hygiene status. By studying both sexes, our work has revealed mechanisms by which the gut microbial community (the microbiome) influences sex hormones, metabolites, and autoimmunity in the NOD model. A focus of our current effort is to dissect the sex chromosome and hormonal mechanisms that regulate the gut microbiome and the function of the gut-associated immune system.

UTI Complexity Results from Diversity at the Bacterial-Host Interface

Scott Hultgren, PhD
Washington University School of Medicine

Our studies blend multiple scientific disciplines elucidating bacterial and host mechanisms that determine the onset, course, and outcome of interactions between uropathogens and host tissues. We have illuminated bacterial mechanisms, intracellular lifestyles, and community behaviors, which play critical roles in urinary tract infection (UTI). We have shown that risk of UTI depends on specific pairing between diverse uropathogens and host susceptibility, with the outcome depending on both bacterial gene carriage and transcriptional responses, and complex host mucosal response networks, governed by disease history and host sex factors. Our work is changing the way UTIs are evaluated and is informing the design of novel vaccines and therapeutics.

Sex Differences in Pathogenesis of Urinary Tract Infection

David A. Hunstad, MD
Washington University School of Medicine

Preclinical modeling of urinary tract infections (UTIs) has been limited to females. We devised a new technique for bladder inoculation with uropathogenic *Escherichia coli* (UPEC) in both male and female mice, enabling first-ever studies of sex differences in UTI. Male mice display striking susceptibility to chronic cystitis, severe pyelonephritis, and renal abscess (rare in female mice); castration or genetic deficiency of the androgen receptor (AR) prevents severe UTI. In female mice, dihydrotestosterone treatment potentiates severe UTI, while the AR antagonist enzalutamide is protective. These data mirror reports of elevated UTI risk in women with polycystic ovary syndrome, a common hyperandrogenic state. Our work illuminates sex influences on urogenital infections and suggests androgen modulation as a therapeutic strategy in recalcitrant or recurrent UTI.

*Estrogen Contributes to Sex Differences in M2-Polarization During Asthma

Aleksander Keselman, PhD
Johns Hopkins University

Asthma exhibits sex differences, affecting mostly boys in childhood and women in adulthood. Alveolar macrophages have emerged as major mediators of allergic lung inflammation. We hypothesized that estrogen enhances the M2 polarization of alveolar macrophages to promote asthma. We found M2-gene expression to be elevated in alveolar and bone-marrow derived macrophages (BMMs) from female mice after challenge with allergen and stimulation with IL-4, respectively. Pretreatment of female BMMs with estrogen receptor ligands enhanced IL-4-induced M2-gene expression. Ovariectomized and LysMCRE ERa flox/floxmice exhibited impaired M2 responses after challenge with OVA. Together these data suggest that sex and hormonal factors contribute to sex differences in macrophage responses during asthma.

This oral presentation will also be a poster presentation.

Sex Differences in Brain Gut Microbiome Interactions

Emeran A. Mayer, MD
University of California, Los Angeles

Even though brain gut microbiome (BGM) interactions play a crucial role in the homeostasis of the organism, few studies have addressed sex-related differences in these interactions. To test this general hypothesis, we performed several studies aimed to characterize BGM interactions in subjects with chronic visceral pain (irritable bowel syndrome [IBS]), with obesity and in healthy controls. Behavioral data, multimodal brain imaging, and microbiome data were obtained in the three groups, and analyses of brain network connectivity, gut microbial community structure, and microbial metabolites were performed. Female IBS subjects showed lower sympathetic nervous system responses, lower HPA axis responses, and greater perceptual responses to an aversive visceral stimulus, compared to males. At the brain level, females showed more prominent sensorimotor structural and functional alterations, while males showed more prominent changes in the salience network. At the microbiome level, significant differences in

plasma levels of several microbial-derived tryptophan metabolites were observed. In obese subjects, significant sex-related differences were seen in anatomical and functional network connectivity involving cortical and subcortical regions, in addition to differences in gut microbial metabolites. The observed sex-related differences within the BGM axis may have implications for personalized treatment approaches.

Incorporating Sex as a Biological Variable in Understanding Brain Development

Margaret McCarthy, PhD
University of Maryland School of Medicine

Most people associate sex differences in brain and behavior with adult circulating hormones that can vary dramatically between males and females. But many adult sex differences are actually established early in development, beginning in utero and extending post-natally, as a result of elevated androgens in males that derive from the fetal testis. This early life programming has enduring effects that are often not manifest until the animal matures and is capable of exhibiting complex motivated and cognitive behaviors. Parsing out the contribution of biology to sex differences in adult behavior is complicated by the impacts of environment and experience, which have accumulated during the maturational period. Study of the immature brain provides an opportunity to more tightly control extraneous variables and thereby more cleanly delineate the impact of nature versus nurture. Our research program has focused on the molecular mechanisms by which early hormone exposure in males differentiates the neuroanatomical and genomic substrate. We have identified novel sources of modulation of synaptogenesis, synaptic pruning, cell genesis, and differentiation. These include unexpected roles for prostaglandins, endocannabinoids, microglia, and other immune cells. We have also found a profound effect of steroid exposure early in life on the epigenome of the brain and speculate this is a mechanism by which sex differences are established and endure. Our work highlights latent variables that may increase the risk of males to developmental neuropsychiatric and neurodevelopmental disorders while protecting females from the same. Supported by R01 DA039062 and R01MH052716.

Sex-Specific Risk for Cardiovascular Dysfunction and Cognitive Decline

Virginia M. Miller, PhD
Mayo Clinic

Women who experience a preeclamptic pregnancy had greater carotid intima-media thickness (CIMT), coronary artery calcification, lower cerebrovascular reactivity, and greater cognitive impairment and cortical atrophy than women who experienced normotensive pregnancies. In healthy, post-menopausal women neither transdermal 17β-estradiol nor oral conjugated equine estrogen affected CIMT during or within 3 years following the menopausal hormone treatment (MHT). Treatment effects were influenced by genetic variants associated within genes of the innate immune system, estrogen metabolism, and APOε4. Understanding the impact of pregnancy outcomes on risk of chronic diseases of aging and the therapeutic potential of MHT to alleviate them helps to inform personalized care of women as they age.

Sex Differences in Pain Management

Anne Z. Murphy, PhD
Georgia State University

Chronic pain is one of the most commonly reported health problems in the United States, affecting approximately 25% of the population. As clinicians advance toward more individualized treatment strategies for pain, the importance of biological sex is increasingly clear. Women have a higher incidence rate of chronic pain conditions, and in particular, those that include an inflammatory component, such as fibromyalgia and osteoarthritis. Morphine has been and continues to be one of the most effective drugs for the treatment of pain. However, preclinical studies have repeatedly demonstrated that morphine is a more effective analgesic in males than in females. Clinical studies examining sex differences in analgesia are more varied. This talk will discuss the various central mechanisms that contribute to the dimorphic effects of morphine.

Sex and Mouse Brain Anatomy during Health and Treatment

Brian Nieman, PhD
The Hospital for Sick Children

The brain undergoes extensive and prolonged development after birth with subtle anatomical differences between males and females. Events that affect this development often also show sex dependence. Children treated for cancer at an early age frequently experience lasting cognitive or behavioral difficulties, with females more susceptible than males. Using three-dimensional imaging to assess anatomy, we have similarly demonstrated sexual dimorphism in brain structure and regional volume changes in the brain after cancer treatment using a mouse model. The role of inflammatory response after cranial radiation, in particular, was investigated and shown to vary by sex.

Sex in Perinatal Brain Damage: Why Can't Boys Be More Like Girls?

Anna Penn, MD, PhD
Children's National Medical Center

Ten percent of all infants in the United States are born preterm, and more extremely preterm infants are being saved, so there is an increasing need to understand the factors contributing to brain damage in these infants. Despite improvements in survival, survivors remain at high risk for cerebral palsy and mental retardation, learning disability, and later psychiatric illness. Even in the absence of obvious structural brain injury, a significant number of infants born very preterm have cognitive deficits and behavioral problems. *Males are at particularly high risk.* A key contributor to this damage may be loss of placental hormones, including sex steroids and other hormones. To understand the placental hormone contribution to perinatal brain injury, and specifically to increased male vulnerability, we are pursuing investigations that range from the development of novel mouse models to use of human infant data. Our overall goal is to understand hormones that contribute to normal neurodevelopment, the effects of their loss following premature birth, and their potential as protective agents.

Sex Stratification for Obesity and Related Traits in African Populations—H3Africa AWI-Gen Study

Michèle Ramsay, PhD
University of the Witwatersrand, Johannesburg

The health and epidemiological transition across Africa is evident in the rising tide of obesity and its impact on hypertension and diabetes. Unlike the developed world, the sex disparity is marked in Africa, with women disproportionately affected. In a multi-center African study across four countries, obesity (BMI≥30) was highest (67%) among women in urban South Africa (42% men) and lowest in rural Burkina Faso (2.2% in women; 1.8% in men), where 31% of women (17% men) are underweight (BMI<18.5). These differences result from complex interactions between fixed (sex, age, genetic variation, biology) and modifiable (behavior, sociodemographic) risk factors. We need effective interventions to ameliorate the impact of obesity on population health.

Sex-Specific Genetic Architecture of the Human Transcriptome

Barbara Stranger, PhD
University of Chicago

Gene regulation may contribute to sexually dimorphic complex traits. We have characterized the role of sex in inter-individual transcriptional variation in tissues of the Genotype-Tissue Expression (GTEx) Project. Our goals are to (a) identify genes and regulatory networks differentially expressed (DE) between sexes, (b) identify and characterize sexually dimorphic expression quantitative trait loci (eQTLs), (c) pinpoint differences in sex-biased eQTL profiles between autosomal and sex chromosomes, and (d) determine the variation of sex biases across tissues and their contribution to disease. We present highlights of this ongoing work.

Sex-Specific Metabolic and Epigenetic Changes in Primary Fibroblasts from Patients with Alzheimer's Disease

Eugenia Trushina, PhD
Mayo Clinic

Complex changes that occur in Alzheimer's disease (AD) patients and the lack of understanding of early disease mechanisms have hindered the development of efficacious therapeutic interventions. Systems biology offers an outstanding opportunity to monitor changes involved in AD development in multiple functionally connected pathways using readily available biofluids or primary cells such as fibroblasts. We applied non-targeted and targeted metabolomics, stable isotope tracers, and next generation sequencing to establish to what extent metabolic and epigenetic changes in fibroblasts from late onset AD patients recapitulate alterations established previously in CSF, plasma, and postmortem brain tissue from individuals with mild cognitive impairment and AD. Our data revealed sex- and disease-specific signatures validating the use of human fibroblasts to study AD mechanisms.

X, Drugs, Rock, an' Roles…

Lauren A. Weiss, PhD
University of California, San Francisco

Sexual dimorphism in common complex genetic disease is extensive; the male predominance of autism spectrum disorders is one example of a longtime observation that remains largely unexplained. Possible genetic mechanisms include a disproportionate role for sex chromosomes, a substantial role for hormones (potentially via effects on gene expression), or different thresholds determining sufficient genetic liability. In addition, it is unknown whether the sex differences in autism are specific to its behavioral or neurodevelopmental features or will be representative of general sexual dimorphism in biology. We designed a study to evaluate evidence supporting these hypotheses utilizing common polymorphism genome-wide association study data.